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These studies established that the biosynthesis of S-(pentachlorobutadienyl)glutathione (PCBG) is catalyzed preferentially by hepatic microsomal glutathione S-transferases. PCBG is further metabolized to the diconjugate 1,4-bis(glutathion-S-yl)-1,2,3,4-tetrachlorobuta-1,3-diene by hepatic cytosolic transferases. Studies on the synthesis of PCBG in the isolated, perfused rat liver showed that PCBG is eliminated in the bile at toxicologically relevant doses. The cysteine analog of PCBG S-(pentachlorobutadienyl)-L-cysteine (PCBC) is a potent nephrotoxin that damages mitochondria. PCBC, which is activated by renal mitochondrial cysteine conjugate β-lyase, inhibits mitochondrial protein, DNA, and RNA synthesis and destroys mitochondrial DNA, although the role of the effects in the observed mutagenicity of PCBC is unclear. Finally, preliminary studies on the intestinal absorption of PCBG indicate that the intact glutathione S-conjugate is absorbed in vivo and is cultured CaCo cells.

14. SUBJECT TERMS
Glutathione S-conjugates, cysteine S-conjugates, nephrotoxicity, hexachlorobutadiene, S-(pentachlorobutadienyl)glutathione, S-(pentachlorobutadienyl)-L-cysteine

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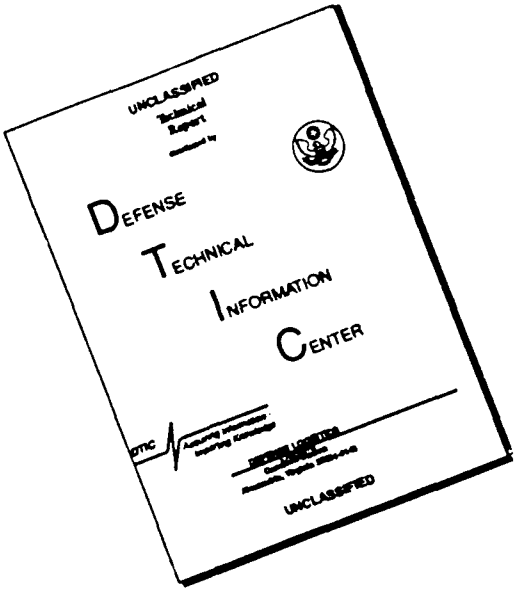

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FINAL TECHNICAL REPORT

Project Title:	Biosynthesis, Physiological Disposition, and Biochemical Effects of Nephrotoxic Glutathione and Cysteine S-Conjugates
Project Number:	AFOSR-86-0302
Principal Investigator:	M. W. Anders
Period Covered:	8/15/86 to 2/14/90

Biosynthesis of S-(pentachlorobutadienyl)glutathione (PCBG): The metabolism of hexachlorobutadiene (HCBD) to PCBG was studied with rat hepatic microsomal and cytosolic fractions. The major product formed was PCBG, which was fully characterized by ^{1}H and ^{13}C NMR and by fast-atom bombardment mass spectrometry; the microsomal glutathione S-transferase was a more efficient catalyst of the formation of PCBG than were the cytosolic transferases. PCBG was metabolized to the diconjugate 1,4-bis(glutathion-S-yl)-1,2,3,4-tetrachlorobuta-1,3-diene by the cytosolic transferases. Although PCBG is nephrotoxic, insufficient diconjugate is available for toxicity studies. This work has been published (Dekant et al., 1988).

The biosynthesis of PCBG was also studied in the isolated, perfused rat liver. HCBD was metabolized to PCBG, which was almost exclusively eliminated in the bile at nonhepatotoxic doses of HCBD; when hepatotoxic doses of HCBD were studied, PCBG was eliminated in both the bile and in the caval effluent. Depletion of hepatic glutathione concentrations decreased PCBG biosynthesis in the perfused liver. This work has been submitted for publication (Gietl and Anders, 1990).

Attempts to study the in vivo biosynthesis of [^{35}S]PCBG from HCBD by labeling the hepatic glutathione pool by giving [^{35}S]methionine failed, because the degree of labeling that could be attained was not sufficient. This strategy, which would have broad applicability, merits further investigation.

Alternative Routes of Reactive Metabolites of PCBG: In order to investigate the bioactivation mechanism of S-(pentachlorobutadienyl)-L-cysteine (PCBC), we sought alternative strategies to generate reactive intermediates of PCBC. Hence we prepared benzyl pentachlorobutadienyl sulfide, the hypothesis being that cytochromes P-450 would metabolize the sulfide to the corresponding hemimercaptal, which would eliminate the same reactive intermediate that is formed by the action of cysteine conjugate ß-lyase on PCBC. The expectation was correct: benzyl pentachlorobutadienyl sulfide was cytotoxic in isolated rat hepatocytes and was metabolized to benzaldehyde by purified,

reconstituted cytochrome P-450$_{PBB}$. The analog tert-butyl pentachlorobutadienyl sulfide, which cannot form a hemimercaptal, was not, as expected, cytotoxic. This work has been published (Veltman et al., 1989). Benzyl pentachlorobutadienyl sulfide is mutagenic in the Ames test and requires activation by cytochromes P-450; this work has been published (Vamvakas et al., 1989).

In other experiments, not yet submitted for publication, we prepared the proreactive intermediate 2-nitrophenyl pentachlorobutadienyl disulfide. Reduction of the disulfide affords pentachlorobutadienylthiol, the putative reactive intermediate formed by the ß-lyase-catalyzed metabolism of PCBC. We are presently attempting to characterize fully the products formed. Such stable, synthetically accessible precursors of reactive intermediates will find utility in exploring the properties of biological reactive intermediates.

Mitochondrial Toxicity of PCBC: Previous studies showed that mitochondrial are the primary targets for nephrotoxic cysteine conjugates. Hence the effect of PCBC on renal mitochondrial protein, RNA, and DNA synthesis was studied. PCBC inhibited mitochondrial protein synthesis, and this effect was blocked by the ß-lyase inhibitor aminooxyacetic acid. Similarly, PCBC inhibited mitochondrial RNA and DNA synthesis, and this effect was also blocked by aminooxyacetic acid. PCBC also damages the mitochondrial genome. In mitochondria incubated with PCBC, 60% of the supercoiled, high-molecular weight mtDNA was degraded to the relaxed circular form and to small fragments. Because this effect was shared with nonmutagenic cysteine conjugates, it probably does not contribute to the nephrocarcinogenicity of HCBD. This work has been published (Banki and Anders, 1989).

Intestinal Absorption of PCBG: In work completed at the end of the period of support, we found that PCBG infused into the bile duct of rats is absorbed largely intact and can be detected in blood; PCBC was also detected in blood, indicating that some hydrolysis does take place. In studies with cultured intestinal CaCo cells, PCBG is actively transported as the intact glutathione S-conjugate. We hope to complete these studies with other funds.

Summary: These studies have provided new information about the biosynthesis of the glutathione conjugate PCBG and about the mitochondrial toxicity of the nephrotoxic cysteine analog PCBC. Preliminary studies indicate that PCBG is absorbed intact from the intestine. A particularly significant accomplishment was the development of proreactive intermediates of cysteine conjugates; these compounds are expected to find utility in investigating cytotoxic and mutagenic glutathione conjugates.

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